

# Assessment of the Association between *Blastocystis* Infection and Irritable Bowel Syndrome

Surapon Surangsrirat MD\*, Luksana Thamrongwittawatpong MD\*,  
Wanit Piyaniran MD\*, Tawee Naaglor BSc\*\*,  
Chananan Khoprasert MSc\*\*\*, Paanjit Taamasri MSc\*\*,  
Mathirut Mungthin MD, PhD\*\*, Saovane Leelayoova, PhD\*\*

\* Division of Gastroenterology and Liver disease, Phramongkutklao Hospital, Ratchawithi Road, Ratchathewi, Bangkok, Thailand

\*\* Department of Parasitology, Phramongkutklao College of Medicine, Ratchawithi Road, Ratchathewi, Bangkok, Thailand

\*\*\* Department of Microbiology, Phramongkutklao College of Medicine, Ratchawithi Road, Ratchathewi, Bangkok, Thailand

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**Objective:** To assess the association between *Blastocystis* infection and irritable bowel syndrome (IBS) in Thai patients, Phramongkutklao Hospital.

**Material and Method:** A case-control study was conducted at Phramongkutklao Hospital, Bangkok, Thailand during 2007-2008. A total of 126 subjects were enrolled into the study. Sixty-six persons were enrolled in the IBS group and 60 persons were enrolled in the control group. Intestinal parasitic infections were determined using wet preparation and formalin-ethyl acetate concentration. Short-term in vitro culture was performed to detect *Blastocystis* sp. Additionally, stool specimens were cultured for pathogenic bacteria. Patients' history and physical examination were also recorded.

**Results:** Of 66 IBS patients, 61 (92.4%) had abdominal pain, 50 (78.1%) had diarrhea, and 14 (21.9%) had constipation. In vitro cultivation revealed that 10.0% and 16.7% were positive for *Blastocystis* sp. in the control and IBS group, respectively. There was no significant difference of the prevalence of *Blastocystis* infection between these 2 groups ( $p = 0.203$ ).

**Conclusion:** *Blastocystis* infection was a common parasitic infection which was predominant in both IBS and control group. The presence of *Blastocystis* sp. in stool did not imply gastrointestinal symptoms in the IBS patients. Further study of subtype characterization of *Blastocystis* sp. in a larger sample size of the case-control study might reveal a possible relationship between *Blastocystis* sp. and IBS patients.

**Keywords:** *Blastocystis* sp, Irritable bowel syndrome (IBS)

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Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal condition characterized by abdominal discomfort, bloating and disturbed defecation with no identifiable physical, radiological or laboratory abnormalities. Rome Committee for the Classification of Functional Gastrointestinal Disorders has defined IBS on the basis of abdominal and bowel symptoms that occur with sufficient frequency in affected patients<sup>(1)</sup>. IBS is a common condition, with the prevalence ranging between 12% and 15% in the

western countries<sup>(2,3)</sup>. IBS is one of the most common reasons for primary care visits. More than 40% of IBS patients have so frequent and severe symptoms which disturb their work and social life<sup>(4)</sup>. The cause of IBS is still uncertain whereas the possible hypotheses are; altered motility of bowel movement, abnormal visceral sensation, post infectious, psychosocial factors, and neurotransmitter interplay to cause symptoms. In developing countries, gastrointestinal infection is frequently found and remains the major problem which probably causes and relates to IBS.

*Blastocystis* sp., a ubiquitous intestinal protozoa, has been reported worldwide especially in the tropical developing countries. The prevalences of *Blastocystis* carriage in healthy asymptomatic

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**Correspondence to:**

Leelayoova S, Phramongkutklao College of Medicine, 315 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand.  
Phone: 0-2354-7600  
E-mail: [s\\_leelayoova@scientist.com](mailto:s_leelayoova@scientist.com)

individuals in developed and developing countries were 10-15% and 30-50%, respectively<sup>(5-7)</sup>. In Thailand, the prevalences of *Blastocystis* carriage in various groups were as high as 10-40% which makes this organism be the most frequently reported protozoa in stool specimens<sup>(7,8)</sup>. Its significance as a pathogen remained controversial for a decade. Several studies have reported *Blastocystis* sp. as a causative agent of gastrointestinal symptoms such as nausea, anorexia, abdominal pain, flatulence and acute or chronic diarrhea in both immunocompetent and immunocompromised hosts<sup>(9-11)</sup>.

It has been hypothesized that *Blastocystis* sp. may play a role in IBS patients. Recently, there have been a few studies that have shown the link between *Blastocystis* sp. and IBS<sup>(12, 13)</sup>. However, the studies showed contradictory results<sup>(13,14)</sup>. Thus, this study aimed to determine the association between IBS and *Blastocystis* infection in the patients who visited Phramongkutklao Hospital, Bangkok, Thailand

## Material and Method

### Study population

A case-control study was conducted at Phramongkutklao Hospital, Bangkok, Thailand. Research protocol was approved by the Ethical Committee of the Royal Thai Army Medical Department. The control and IBS group were those who attended the gastroenterology clinic during 2007-2008. Those who were not diagnosed of IBS were the control group. IBS patients were defined using Rome II criteria<sup>(15)</sup>. The Rome II criteria used was as followed; patients had at least 12 weeks or more, need not be consecutive, in the preceding 12 months of abdominal discomfort or pain which had two of three features: relieved with defecation; and/or onset associated with a change in frequency of stool; and/or onset associated with a change in form of stool. The exclusion criteria were as followed; alarming symptoms such as weight loss, anemia, hematochezia, fever, chronic diseases e.g. chronic kidney disease, diabetes, coronary artery disease, having metronidazole treatment within 1 month. Sixty-six patients were enrolled in the IBS group and 60 patients were enrolled in the control group. Information of their medical history and physical examination were obtained.

### Stool collection and examination

Stool specimens were examined for intestinal parasitic infections and cultured for *Blastocystis* sp., at the Department of Parasitology, Phramongkutklao

College of Medicine. Examination for intestinal parasites was done by wet smear preparation and formalin/ethyl-acetate concentration technique. Short-term *in vitro* cultivation was performed for the detection of *Blastocystis* sp. using Jones' medium supplemented with 10% horse serum<sup>(16)</sup>. The cultures were incubated at 37°C for 48-72 hours and then examined under a light microscope. Positive *Blastocystis* sp. was defined as identification of any form of *Blastocystis* sp., i.e., amoeboid, vacuolar, avacuolar, multivacuolar, granular and cystic forms by short-term *in vitro* cultivation. Each stool specimen was also examined for *Cryptosporidium* spp and microsporidia using modified acid-fast and gram-chromotrope staining, respectively. Stool samples were also transferred in transport media and cultured for bacterial pathogens using conventional cultivation. These bacterial pathogens were then identified by biochemical methods at the Department of Microbiology, Phramongkutklao College of Medicine. All samples were tested for common bacterial pathogens that may cause gastrointestinal symptoms such as *Salmonella* spp, *Shigella* spp, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Vibrio cholera*.

### Statistical analysis

Results were expressed as the mean  $\pm$  SD for continuous variables (e.g., age) and number (percentage) for categorical data (e.g., sex, stool culture, diarrhea, etc). Means of different groups were compared using the independent sample t-test. Pearson Chi-square test and Fisher's exact test were also used for comparing proportional data. P-value < 0.05 was considered statistically significant. Statistical interpretation of data was performed by using Stata/SE for Windows version 9.2 (Stata Corp LP, College Station, TX).

## Results

Of 66 IBS patients, 41 (62.1%) were female. The mean age of the IBS group was  $49.5 \pm 13.8$  years. The bowel habit was described as abdominal pain (61, 92.4%), diarrhea (50, 78.1%) and constipation (14, 21.9%). Consistency of stool varied from formed (50, 75.8%), soft (15, 22.7%), and watery (1, 1.5%). The control group consisted of 30 males and 30 females with the mean age of  $45.3 \pm 13.9$  years. Consistency of stool varied from formed (42, 70%), soft (18, 30%), and watery (0, 0%).

There was no significant difference in age between both groups ( $p = 0.6$ ). There were more women than men in the IBS group without statistical significance ( $p = 0.61$ ). Abdominal pain was found

**Table 1.** Prevalence of parasitic infection in Irritable bowel syndrome (IBS) and control patients

Organisms	IBS (n = 66)	Control (n = 60)
<i>Blastocystis</i> sp.	11 (16.7%)	6 (10%)
Microsporidia spore	1 (1.5%)	0 (0%)
<i>Ascaris</i> egg	0 (0%)	1 (1.7%)
<i>Entamoeba coli</i>	0 (0%)	1 (1.7%)
<i>Salmonella</i> group B	2 (3.0%)	3 (5%)
<i>Salmonella</i> group C	1 (1.5%)	0 (0%)
<i>Salmonella enteritidis</i>	1 (1.5%)	0 (0%)

higher in the IBS group (92.4%) than in the control group (26.7%) ( $p < 0.001$ ). Other underlying diseases, smoking status, alcohol drinking, and characteristic of stool appearance were not different between both groups ( $p > 0.5$ ).

The prevalence of intestinal parasitic and bacterial infections in the IBS and control group was shown in Table 1. Microscopic examination revealed that 15% (6 of 66) and 12.5% (5 of 60) of the IBS and control group were positive for *Blastocystis* sp. Short-term *in vitro* culture showed more sensitive than microscopic examination for the detection of *Blastocystis* sp. Using *in vitro* culture, *Blastocystis* sp. in the IBS group was 16.7% (11 of 66) while 10.0% (6 of 60) were detected in the control group ( $p = 0.203$ ). There was no significant difference between the IBS patients (2 of 23) and the control group (5 of 23) in term of diarrhea symptom ( $p = 0.414$ ).

## Discussion

In the past, pathogenic potential of *Blastocystis* sp. remains uncertain. Convincing evidence of its pathology has been shown by several groups of researchers. Pathological mechanisms caused by *Blastocystis* sp. were demonstrated as followed; elicitation of toxic-allergic reactions such as chronic urticaria<sup>(17)</sup>; degradation of human secretory IgA by cysteine proteases from zoonotic isolates (*Blastocystis ratti*) which localized to the parasite central vacuole<sup>(18,19)</sup>; changes of epithelial permeability by inducing apoptosis of host intestinal cells and disruption of the epithelial barrier function<sup>(20)</sup>, modulation of the host immune response and cytokine releasing from colonic epithelial cells<sup>(19)</sup>. Moreover, the existence of amoeboid form of *Blastocystis* sp. was observed exclusively in the symptomatic cases<sup>(21)</sup>. In symptomatic patients, clinical manifestations of

blastocystosis have been associated basically with abdominal pain, diarrhea, constipation, fatigue, skin rash, nausea, anorexia, vomiting, flatulence, weight loss, lassitude and dizziness<sup>(22, 23)</sup>.

IBS is a complex and heterogeneous group of disorders in which gastrointestinal inflammation is one of the proposed mechanisms of pathogenesis. *Blastocystis* sp. has been proposed as one of possible causes in IBS<sup>(5,12,13,24)</sup>. In addition, levels of IgG antibody to *Blastocystis* sp. were increased significantly in the patients with IBS compared with asymptomatic control group<sup>(12)</sup>. At present, a few case-control studies were conducted to demonstrate the association of *Blastocystis* infection and IBS. To compare the results among these studies, detection of *Blastocystis* sp. in stool specimens had to be conducted using standard methods. In addition, microbiological analyses to rule out other infections that could cause gastrointestinal symptoms need to be performed. Our previous study demonstrated higher sensitivity of a short-term *in vitro* culture of *Blastocystis* sp. than those of formalin ethyl-acetate sedimentation and trichrome staining<sup>(16)</sup>. To date, short term *in vitro* cultivation has basically been used and accepted as gold standard for the detection of *Blastocystis* sp. due to its high sensitivity and specificity. This study confirmed the usefulness of short-term *in vitro* culture of stool for *Blastocystis* sp. which was also reported by several studies<sup>(13,14,16)</sup>. Thus, the results of this study were compared only with other previous studies of which *in vitro* cultures of stool for *Blastocystis* sp. were used as a sensitive method of diagnosis. In this study, *Blastocystis* sp. was a predominant intestinal parasitic infection in both IBS and control groups (17.5% vs. 12.5%). However, the prevalences of *Blastocystis* sp. in the case and control group were not significantly different. Recently, a cohort study of *Blastocystis* sp. in Thai IBS conducted in Bangkok showed the same results as ours which IBS patients and controls had no significant difference of harboring *Blastocystis* sp. (13.6% vs. 12%)<sup>(14)</sup>. In contrast to recent study by Yakoob et al (2004), using *in vitro* culture of stool specimens, 46% of IBS patients harbored *Blastocystis* sp. which was significantly higher than those seen in the control<sup>(13)</sup>. Molecular studies suggest that specific subtypes of *Blastocystis* sp. seem to cause intestinal disorders. Some specific subtypes of *Blastocystis* sp. such as subtype 1 and subtype 3 were shown to be associated with symptomatic cases<sup>(11)</sup>, while subtype 2 was found in asymptomatic carriers<sup>(25)</sup>. However, recent study contradicted this finding. The study by Dogruman et al, (2009) showed

no statistical significance of the distribution of *Blastocystis* sp. subtypes between symptomatic and asymptomatic individuals<sup>(26)</sup>. Taken together, *Blastocystis* sp. causes IBS or not is still unclear. It can be postulated that the clinical outcomes of *Blastocystis* sp. infection in humans are not only determined by the presence of organism itself, but probably also associated with host genetics, immune status or/and intrinsic factors<sup>(22,26)</sup>. Further investigation may help explain why individuals harbored the same subtype showed different symptoms.

The predominant symptoms in the IBS group of this study were abdominal pain and diarrhea, respectively. It has been demonstrated that proteases were responsible for visceral pain and diarrhea in IBS patients. Increased fecal serine protease activity, a colonic luminal factor which impaired colonic permeability was detected in diarrheic IBS patients<sup>(27)</sup>. These proteases could directly stimulate sensory neurons and generate hypersensitivity symptoms through the activation of protease-activated 2 (PAR2) receptor<sup>(28)</sup>. This could explain the case of symptomatic *Blastocystis* sp. infection, cysteine protease secreted by *Blastocystis* sp. might account for abdominal pain in the IBS/*Blastocystis* patients. Variation of levels of cysteine protease between subtypes 5 and 7 of *Blastocystis* sp. was also demonstrated<sup>(29)</sup>. Further studies of cysteine protease in other subtypes of *Blastocystis* sp. might reveal its significance in symptomatic cases.

In the present study, stool culture for bacteria revealed *Salmonella* group B, *Salmonella* group C and *Salmonella enteritis* in the IBS patients. This was not different to those detected in the controls which *Salmonella* group B was identified in 3 patients. Epidemiologic studies examining the rate of IBS following bacterial gastroenteritis have been observed. One important observation is that patients with preexisting IBS may be more likely to present to their physicians with bacterial gastroenteritis compared with those without IBS<sup>(30)</sup>. Thus, IBS following bacterial gastroenteritis may have been overestimated. However, this study did not find any significant difference of pathogenic bacteria between the cases and controls. It has also been suggested that treatment of *Blastocystis* sp. should be considered especially when no pathogens have been ruled out in gastrointestinal illness.

In conclusion, *Blastocystis* sp. is a very common intestinal infection in Thai population and its prevalence was not significantly different in IBS and control groups. Further investigation need to be done

with a larger sample size of patients. In addition, development of validated biochemical and/or molecular tools is crucial for differentiation of pathogenic and non-pathogenic organism.

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## การประเมินความสัมพันธ์ของการติดเชื้อ *Blastocystis* กับภาวะ Irritable Bowel Syndrome

สุรพล สุรางค์ศรีรัตน์, ลักษณะ อารงวิทวัสพงศ์, วานิช ปิยนรินทร์, ทวี นาคหล่อ, ชนนานันท์ ขอบประเสริฐ, ปานจิต ธรรมศรี, มัทริศ มุ่งถิ่น, เสาวนีย์ ลีละยูวะ

**วัตถุประสงค์:** ศึกษาหาความสัมพันธ์ของการติดเชื้อบลาสโตซิสติส (*Blastocystis* sp) กับภาวะ irritable bowel syndrome (IBS) ในผู้ป่วยไทยที่มารับการรักษาที่โรงพยาบาลพระมงกุฎเกล้า

**วัสดุและวิธีการ:** การศึกษาชนิด case-control ในโรงพยาบาลพระมงกุฎเกล้า ในปี 2550-2551 มีผู้ป่วยจำนวน 126 ราย ที่ยินยอมเข้าร่วมในการศึกษา โดยมีผู้ป่วยในกลุ่ม IBS จำนวน 66 ราย และกลุ่มควบคุมจำนวน 60 ราย การตรวจหาปรสิตในลำไส้ใช้วิธีการตรวจหาเชื้อด้วยกล้องจุลทรรศน์โดยใช้วิธี wet preparation และ formalin-ethyl acetate concentration ส่วนการตรวจหาเชื้อ *Blastocystis* sp ทำโดยการเพาะเชื้อในหลอดทดลองโดยใช้ Jones' medium นอกจากนั้นได้เพาะเชื้อเพื่อตรวจหาแบคทีเรียที่ก่อโรคในลำไส้ พร้อมกับทำการบันทึกประวัติคนไข้ และผลการตรวจร่างกาย

**ผลการศึกษา:** ผู้ป่วย IBS ทั้งสิ้น 66 ราย มีอาการปวดท้อง 61 ราย (92.4%) ท้องเดิน 50 ราย (78.1%) และท้องผูก 14 ราย (21.9%) ตรวจพบเชื้อ *Blastocystis* sp ในกลุ่มควบคุม 10.0% และกลุ่ม IBS 16.7% โดยไม่มีความแตกต่างทางสถิติ ( $p = 0.203$ )

**สรุป:** *Blastocystis* sp เป็นเชื้อที่ตรวจพบมากที่สุดในกลุ่มควบคุม และกลุ่ม IBS การตรวจพบเชื้อ *Blastocystis* sp ในอุจจาระไม่ได้บ่งบอกเสมอไปว่าอาการผิดปกติในระบบทางเดินอาหารที่เกิดขึ้นมีสาเหตุมาจากเชื้อ *Blastocystis* sp การศึกษาเพิ่มเติมเพื่อหา subtype ของเชื้อ *Blastocystis* sp ในทั้งกลุ่มควบคุม และกลุ่ม IBS ที่มีจำนวนผู้ป่วยที่มากขึ้น อาจสามารถอธิบายความสัมพันธ์ของเชื้อ *Blastocystis* sp กับผู้ป่วย IBS ได้

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